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Autoreactive T-cells in Goodpasture's syndrome recognize the N-terminal NC1 domain on α3 type IV collagen

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Autoreactive T-cells in Goodpasture's syndrome recognize the N-terminal NC1 domain on α3 type IV collagen. Goodpasture's syndrome is mediated by immunopathogenic autoantibodies to the α3 NC1 domain of type IV collagen. It is not known whether collaborating T-cells participate in this autoreactive response. Here we describe the first T-cell clone isolated from a Goodpasture patient autoreactive to α3 type IV collagen of glomerular basement membrane. To investigate cellular autoreactivity, T-cells from Goodpasture patients or controls were isolated and stimulated by purified native or recombinant type IV collagen proteins and synthetic oligopeptides. Cell surface markers, the T-cell receptor repertoire, and MHC-restriction were analyzed. T-cell clones specific for the α3(IV) NC1 domain were established in two Goodpasture patients, but not in controls. One of the three $CD8^+$ T-cell clones was characterized further. It was MHC class I restricted (HLA-A11) and expressed the T-cell receptor Vβ 5.1. chain. This clone specifically recognized a motif at the N-terminal area of the α3(IV) NC1 domain (AA 51 to 59: GSPATWTTR). We conclude that autoreactive T-cells exists in Goodpasture patients and may play a crucial role in the inflammatory process. T-cell clones are autoreactive to the α3(IV) NC1 domain. At least for one of the clones, the T-cell epitope is different from the putative antibody-binding site.

Goodpasture's syndrome or anti-basement membrane antibody mediated disease is an autoimmune disorder characterized by the production of IgG autoantibodies against type IV collagen of glomerular or alveolar basement membrane (GBM) [1]. Their pathogenic relevance was clearly demonstrated by transfer experiments, in which antibodies eluted from diseased human kidneys were able to induce glomerulonephritis in squirrel monkeys [2]. In addition, the disease may recur in human allografts, if circulating antibodies are still present in the course of transplantation [3]. These anti-GBM antibodies are specifically targeted to the C-terminal α3 NC1 domain of type IV collagen, which was recently established as the Goodpasture autoantigen [4]. Type IV collagen is the major constituent of mammalian basement membranes. It is composed of six genetically distinct α(IV) chains (α1-α 6) [5, 6]. The existence of the α3(IV) chain was verified by molecular cloning of a cDNA that encodes the NC1 domain (232 residues) and a segment of the triple helical domain [7]. The Goodpasture epitope, the combining site for the pathogenic autoantibodies, was localized to the carboxyl-terminus of the NC1 domain of the α3(IV) chain, encompassing the last 36 residues as the primary interaction site [8].

Similar to other autoimmune disorders, such as in myasthenia gravis, the B-cell autoantibody response is thought to result from a disordered T-cell immunoregulatory control [9]. However, it is not known whether or not autoreactive T-cells also play a pathogenic role in Goodpasture's syndrome. Indirect evidence derives from the detection of increased numbers of T-cells (both CD4 and CD8) in the glomeruli of patients with crescentic anti-basement membrane glomerulonephritis [10]. Furthermore, the strong association of HLA-DR and -DQ phenotypes with this disease supports a role for autoreactive T-lymphocytes [11].

It was the aim of the present study to investigate cellular immunity in Goodpasture's syndrome. We started to analyzed T-cell immune responses in affected patients by using the C-terminal NC1(IV) domain of type IV collagen from human glomerular basement membrane as a candidate autoantigen. Potentially autoreactive T-cells were characterized.

Methods

Antigens

The globular domain of human basement membrane type IV collagen was prepared as described previously [12]. Briefly, glomerular (GBM), human placental (PBM) and intestinal (IBM) basement membrane preparations were extracted with 6 M guanidine-HCl, 0.05 M Tris-HCl (pH 7.5) for 12 hours and subsequently digested with bacterial collagenase (Boehringer, Mannheim, Germany) for 24 hours at 37°C. After passage through a DE 52 anion exchange column (2.5 × 40 cm), unbound material was pooled, concentrated and applied to a Bio-Gel A-1.5 m (BioRad, Munich, Germany) molecular sieve column. The purity of the material was confirmed by SDS-PAGE and immunoblotting against anti-type IV collagen α1 chain antibody (Ab), anti-type IV collagen α3 chain Ab, as well as by human Goodpasture sera. Bovine α1(IV), α3(IV), and α5(IV) collagens were prepared according to previously described methods [8]. Other antigens used in the study were type IV collagen NC1 from EHS mouse

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sarcoma, tetanus toxoid (Behringwerke Marburg, Germany), rat tail collagen type I and human placenta collagen type VIII (Sigma Chemical Co., St. Louis, MO, USA). All these antigens were used in a final concentration of 10^{-5}g/ml in all tests. Recombinant NC1(IV) fusion proteins [rα1 (IV) NC1, rα2 (IV) NC1, rα3 (IV) NC1, rα4 (IV) NC1, and rα5 (IV) NC1] were further used as antigens. Among the recombinant peptides two different rα3(IV) proteins were analyzed: (1.) A truncated rα3(IV) protein lacking part of the N-terminal NC1(IV) domain (deletion of the first 14 amino acids of the NC1 domain = 218 residues), (2.) An elongated rα3(IV) NC1 protein (232 residues of the complete NC1 domain plus 12 residues of the triple helical domain). Plasmid construction, expression and purification were reported previously [13]. Briefly, cDNA encoding the NC1 domains of human type IV collagen were expressed in *E. coli* as fusion proteins using the pDS-MCS vector. Purity of expressed proteins was determined on 5 to 22% SDS-PAGE gradient gels using Coomassie Blue staining. In all tests the recombinant proteins were used in concentrations of 10^{-5}, 10^{-6}, and 10^{-7}g/ml. Oligopeptides from the NC1(IV) domain of human α3(IV) type IV collagen, prepared and purified as described previously [8], were screened for the ability to induce T-cell proliferation. Furthermore, HLA-A11 specific motifs were subsequently synthesized and tested in final concentrations of 10^{-8}, 10^{-9} and 10^{-10}g/ml in all assays.

Human type IV collagen NC1(IV)-specific T-cell lines and clones

Mononuclear cells (PBMC) were obtained from peripheral blood by Ficoll-Hypaque (Pharmacia LKB Biotechnology, Milwaukee, WI, USA) density gradient centrifugation. Donors included two patients with Goodpasture's syndrome (B.F., HLA-phenotype, A2 A11 B7 Bw62 Bw6 Cw3 DR2 DRw15 DRw8 DRw52 DQw1; and E.V., HLA-phenotype, A2 A19 B7 B21 Bw4 Bw6 DR2 DR4 DRw 52 DQw2), three patients with anti-GBM antibody negative, ANCA positive crescentic glomerulonephritis (P.F., B.N., K.S.) and two healthy volunteers (M.S., O.P.). Both patients with Goodpasture's syndrome presented with rapidly progressive glomerulonephritis and pulmonary hemosiderosis. Light microscopy showed diffuse glomerulonephritis with endocapillary proliferation, tubulointerstitial disease and glomerular crescent formation in 80% (B.F.) or in 75% (E.V.), respectively. Linear staining for IgG could be demonstrated in both patients. Both received plasma exchange (for 5 days) combined with steroids (250 mg methyl-prednisolone for 3 days, followed by 1 mg/kg/body wt) and cyclophosphamide (1.5 mg/kg/body wt). Cells were obtained three to four weeks after the initial presentation of symptoms. The lymphocytes were thus characterized shortly after the initiation of therapy. They were cultured in RPMI 1640 (Gibco BRL Life Technologie, Eggenstein, Germany) containing L-glutamine, 25 mM Hepes (Gibco), 100 U/ml penicillin G, 100 μg/ml streptomycin (Gibco), 10% pooled human AB serum (supplied by BRK, Nürnberg, Germany) and 10 μg/ml human GBM type IV collagen NC1(IV) at 37°C in 5% CO_2. In order to remove cell debris cells were collected and separated by Ficoll density gradient on day 10 and 21. They were then restimulated with autologous irradiated (30Gy) PBMC (5×10^4 cells/well) and 10 μg/ml NC1 type IV collagen. After three days of culture 5 U/ml of human rIL-2 (Eurocetus, Frankfurt, Germany) were added. Proliferative response with or without 10 μg/ml NC1(IV) was tested by ^{3}H-thymidine incorporation. NC1(IV)-specific T-cell lines were purified and cloned by limiting dilution. Cell numbers ranged from 10 to 0.5 per well. Autologous irradiated PBMC (5×10^4/well), 10 μg/ml NC1(IV) and 50 U/ml human rIL-2 added after three days of culture were used for stimulation. Cloned cells were washed, split and tested again for their proliferative response to NC1(IV).

Proliferation assay

Antigen induced proliferation was examined by ^{3}H-thymidine incorporation assay after 72 hours of culture. Prior to testing, cells were cultured for two to three days without NC1(IV), rIL-2 or autologous irradiated PBMC. All antigens (that is, proteins, oligopeptides and nonamers) used in the study were coincubated with autologous irradiated lymphocytes, cultured for 72 hours, and then tested.

For the analysis of MHC restriction, antigen-presenting cells from healthy donors with known HLA-types and MHC class I specific mAb (anti-HLA-A2, anti-HLA-A11, and anti-HLA-B7; Pasteur Merieux S.V., Lyon, France) were used. Cells were harvested onto glass fibre filters using the PHD cell harvester (Cambridge Technology Inc., Watertown, MA, USA). The incorporated radioactivity was assessed by liquid scintillation counting. For better comparison, results are expressed as stimulation index (SI = cpm cells plus antigen/cpm cells without antigen).Positive result range from 7.5 to 19.5 $\times$ 10^3 cpm, negative results range from 0.5 to 1.5 $\times$ 10^3 cpm.

T-cell phenotype analysis

Cells (1×10^5) were stained with the following FITC- or Phycoerythrine (PE)-conjugated mouse anti-human $F(ab')_2$ monoclonal antibodies at 4°C for 30 minutes: CD4/CD8; CD45 RA; CD45 RO; CD2; and α/β T-cell receptor (Immunotech SA, Marseille, France). Fluorescence was measured using the FACScan® (Becton Dickinson & Co., Heidelberg, Germany). T-cell receptor Vβ chain nucleotide sequence was determined by PCR amplification of cDNA as previously described [14]. Briefly, oligo-(dT)-primed cDNA was sythesized from 1 μg of extracted RNA (Quick Prep® mRNA purification kit; Pharmacia LKB, Milwaukee, WI, USA). For PCR amplification of T-cell receptor variable regions 28 different Vβ oligonucleotide primers were used (Vβ 1, Vβ 2, Vβ 3, Vβ 4, Vβ 5.1, Vβ 5.2 to 3, Vβ 6.1 to 3, Vβ 6.7, Vβ 7, Vβ 8.1 to 2, Vβ 9, Vβ 10, Vβ 11, Vβ 12, Vβ 12aE, Vβ 13.1, Vβ 13.2, Vβ 14, Vβ 15, Vβ 16, Vβ 17, Vβ 18, Vβ 19, Vβ 20, Vβ 21, Vβ 22, Vβ 23, Vβ 24). The cDNAs were amplified using *Taq* polymerase (Boehringer) and standard reaction conditions according to suggestions of the manufacturer (Perkin-Elmer Cetus Corp., Norwalk, CT, USA). Thirty-five PCR cycles were performed (denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min). The amplified products were subjected to electrophoresis on 1.5% agarose gel and transblotted onto nitrocellulose membranes. Blots were hybridized with ^{32}P-labeled TCRβ constant region-specific oligonucleotide probes. TcR insert DNA was subcloned into plasmid vector pTZ18R (Pharmacia, Freiburg, Germany) and sequenced by the chain termination technique using universal, reverse and TCR V and C element-specific primers.

Results

Two patients with Goodpasture's syndrome (B.F., E.V.), three with anti-GBM antibody negative, ANCA positive, crescentic

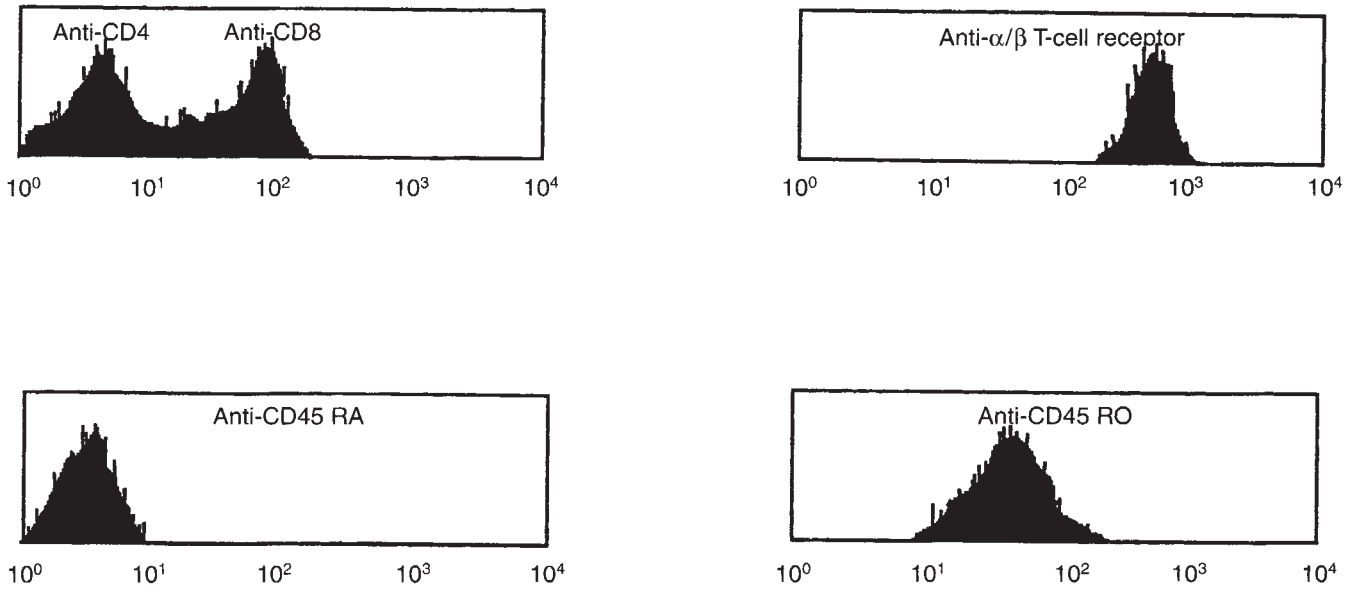

Fig. 1. *Flow histogram demonstrating cell surface expression of CD8, CD45R0 and α/β T-cell receptor on T-cell clone BF-13.* Fluorescence was measured after labeling with monoclonal FITC- or Phycoerythrine conjugated mouse anti-human F(ab') monoclonal antibodies at 4°C for 30 minutes: CD4/CD8; CD45RA; CD45R0; and α/β T-cell receptor (Immunotech SA, Marseille, France). Fluorescence was measured using the FACScan® (Becton Dickinson & Co., Heidelberg, Germany).

glomerulonephritis (P.F., B.N., K.S.) and two healthy volunteers were screened for T-cells, which proliferate in response to the C-terminal NC1 domain of GBM type IV collagen. Antigen restricted T-cell lines could only be established from patients with Goodpasture's syndrome. The stimulation index (SI) in their proliferation assay varied between 2.8 and 10.5 (data not shown). In contrast, no T-cells responding to NC1(IV) could be found in patients with crescentic glomerulonephritis due to ANCA positive systemic necrotizing vasculitis or in healthy volunteers.

Characterization of NC1(IV)-specific T-cell clones (TCC)

T-cells responding to NC1(IV) were further cloned by limiting dilution. Proliferating cells were characterized according to their surface marker proteins, their T-cell receptor, and their MHC restriction. From the two patients with Goodpasture's syndrome three different T-cell clones were phenotyped by FACS. They all were $CD8^+$, $CD45R0^+$, and TCR α/β^+ (Fig. 1). Cells respond to stimulation by the glomerular NC1(IV) domain and to the recombinant α3(IV) NC1 protein (Table 1). The one with the best stimulating response, T-cell clone BF-C13, was characterized further. Cloning and DNA-sequencing of the amplified T-cell receptor Vβ-chain gene indicated the expression of the Vβ 5.1 chain by the isolated T-cell clone (Fig. 2). Proliferative response to antigen was most active in association with the class I allel HLA- A11 (Fig. 2). It was reduced by preincubation with an anti-HLA-A11 specific antibody in a dose-dependent matter. In contrast, anti-HLA-A2 and anti-HLA-B7 were without effect.

T-cell epitope

The established T-cell clone (BF-C13) was tested for responsiveness to various antigens. Tetanus toxoid, collagen type I and collagen type VIII were used as negative controls. NC1(IV) from human placenta, human small intestine and mouse EHS sarcoma cell line [α1(IV) and α2(IV) chain] were used in comparison to NC1(IV) from GBM [α1(IV) to α6(IV) chain, Figure 3A]. Recombinant NC1(IV) domains of α1(IV), α2(IV), α3(IV), α4(IV), and α5(IV) proteins were also used as antigens to clarify T-cell specificity. Proliferation could only be induced by the full length recombinant α3(IV) NC1 protein (Fig. 3b, $N = 5$, 10^{-6} and 10^{-7} g/ml). The truncated recombinant α3(IV) NC1 protein (218AA) stimulated the TCC in a lesser degree only when using higher concentrations (10^{-4} and 10^{-5} g/ml, Fig. 4A). When we used biochemically-purified bovine α3(IV) NC1 with great homology to human α3(IV) NC1, we tried to prove the results obtained with recombinant proteins. T-cell proliferation was stimulated in a minor degree (SI 3.5) when using higher concentrations (10^{-5} g/ml). Bovine α1(IV) and α4(IV) chains did not stimulate the

Table 1. Patient screening for T-cells

	BF C13	BF C20	EV C18	PF	BN	KS
∅	1.4 ± 0.7	1.3 ± 0.8	1.0 ± 0.4	0.9	0.81	0.9
Collagen I	1.2 ± 0.9	1.1 ± 1.0	1.1 ± 0.8	0.87	0.93	0.94
NC1(IV)	15.8 ± 4.1	12.5 ± 5.7	10.7 ± 3.1	0.95	0.84	0.92
α1(IV)	0.9 ± 0.7	1.0 ± 0.9	1.3 ± 1.1	nd	nd	nd
α3(IV)	14.4 ± 3.3	10.7 ± 2.8	7.9 ± 2.1	nd	nd	nd
α5(IV)	1.2 ± 1.3	1.4 ± 0.8	1.1 ± 0.8	nd	nd	nd

Two patients with Goodpasture's syndrome (B.F., E.V.), and three with anti-GBM antibody negative, ANCA positive, crescentic glomerulonephritis (P.F., B.N., K.S.) were screened for T-cells, which proliferate in response to the C-terminal NC1(IV) domain of GBM, in response to collagen type I, and to the recombinant α1(IV) NC1, α3(IV) NC1, and α5(IV) NC1 proteins. T-cell proliferation was examined by ^{3}H-thymidine incorporation assay after 72 hours of culture. The incorporated radioactivity was assessed by liquid scintillation counting. Results are expressed as mean cpm × 10^3 ± SD of triplicate culture ($N = 5$). No T-cells responding to the antigens tested could be established in patients with crescentic glomerulonephritis due to ANCA positive systemic vasculitis. In these patients T-cell stimulation by recombinant proteins has not been done (nd).

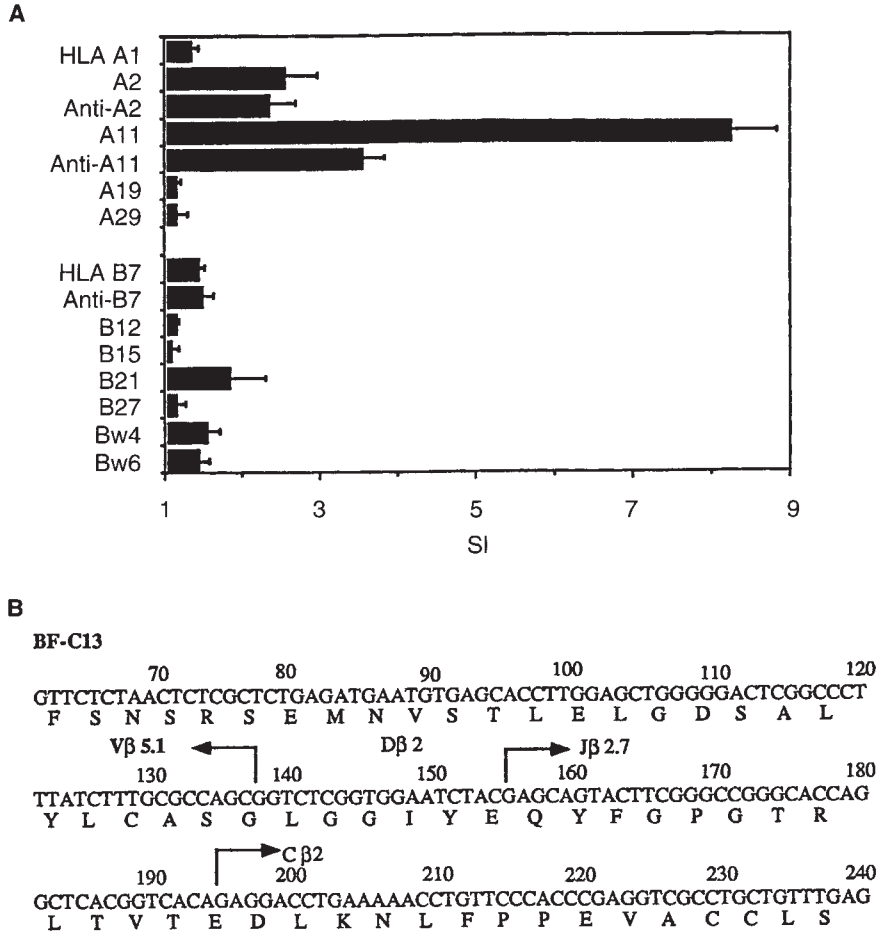

Fig. 2. *MHC-class I restriction and T-cell receptor β chain nucleotide and deduced aminoacid sequence of the T-cell clone BF-C13.* (**A**) For the analysis of MHC restriction, antigen-presenting cells from healthy donors with known HLA-types and MHC class I specific mAb (anti-HLA-A2, anti-HLA-A11, and anti-HLA-B7; Pasteur Merieux S.V., Lyon, France) were used. The proliferation was evaluated by ^{3}H-thymidine incorporation assay after 72 hours of culture. Proliferative response was reduced by preincubation with an anti-HLA-A11 specific antibody in a dose-dependent matter. In contrast, anti-HLA-A2 and anti-HLA-B7 were without effect. (**B**) T-cell receptor Vβ chain nucleotide sequence was determined by PCR amplification of the cDNA. For the PCR amplification of T-cell receptor variable regions 28 different Vβ oligonucleotid primer pairs were used.

TCC (Fig. 3C). These data support the conclusion that the T-cell epitope of the established autoreactive T-cell clone is located on the α3(IV) chain of type IV collagen. Synthetic oligopeptides and a series of nonamer peptides with HLA-A11 specific motifs were used to locate the T-cell epitope for this clone. A proliferative response could be induced with the N-terminal α3(IV) NC1 peptide GLKGKRGDS*GSPATWTTR* (42 to 59, final concentration: 10^{-9}g/ml), whereas the other peptides were unable to stimulate the TCC (Fig. 3C). This observation was further confirmed by using the HLA-A11 specific nonamer 51 to 59 *GSPATWTTR* (final concentration: 10^{-10}g/ml) that also induced a T-cell response. The other peptides tested (57 to 65: TTRGFVFTR; 88 to 96: FLFVQGNQR; 101 to 111:DLGTLGSCLQR; 121 to 131: NVNDVCNFASR; 145 to 154: PMNMAPIIGR; 247 to 255: WLASLNPER; 250 to 259: SLNPERNFRK; 264 to 272: TVKAGELEK) were without effect.

Discussion

Production of either antibodies that react with host tissue or immune effector T-cells that are autoreactive to self-peptides characterize most autoimmune diseases. T-cells have been shown to be pathogenically relevant in multiple sclerosis [15], autoimmune thyroid disease [16], and rheumatoid arthritis [17]. In myasthenia gravis autoantibodies to the acetylcholine receptor produced by B-cells are of pathogenic relevance. However, disordered T-cell function appears to be of critical importance in this disease, too [18]. Glomerulonephritis in autoimmune Goodpasture's syndrome is also caused by pathogenic relevant autoantibodies that are directed to the α3(IV) NC1 domain of type IV collagen. Whether or not cellular immunity plays a role in the pathogenesis of this disease has not been investigated. Evidence for a critical role of cell mediated immunity also in Goodpasture's syndrome can be deduced from experimental animal models. For example, bursectomized chickens immunized with heterologous GBM in adjuvant developed nephritis even in the absence of detectable autoantibodies [19]. In addition, the disease could be induced in naive syngeneic SC chickens simply by passive transfer of T lymphocytes [20]. Furthermore, in a rat model of experimental anti-GBM glomerulonephritis activation of the cell-mediated immune system was evidenced by delayed type hypersensitivity to the immunizing antigen [21]. The development and severity of the disease in this model were not associated with antibody production alone. A role for T-cells in this particular and other animal models [22] is further supported by immunohistochemical demonstration of mononuclear cell-infiltrates in diseased kidneys. Comparable infiltrates have also been shown in human Goodpasture's syndrome. Taken together, these data strongly suggest a role for autoreactive T-cell in this autoantibody mediated disease.

In our study, mononuclear cells were obtained from peripheral blood of Goodpasture patients and stimulated by human GBM

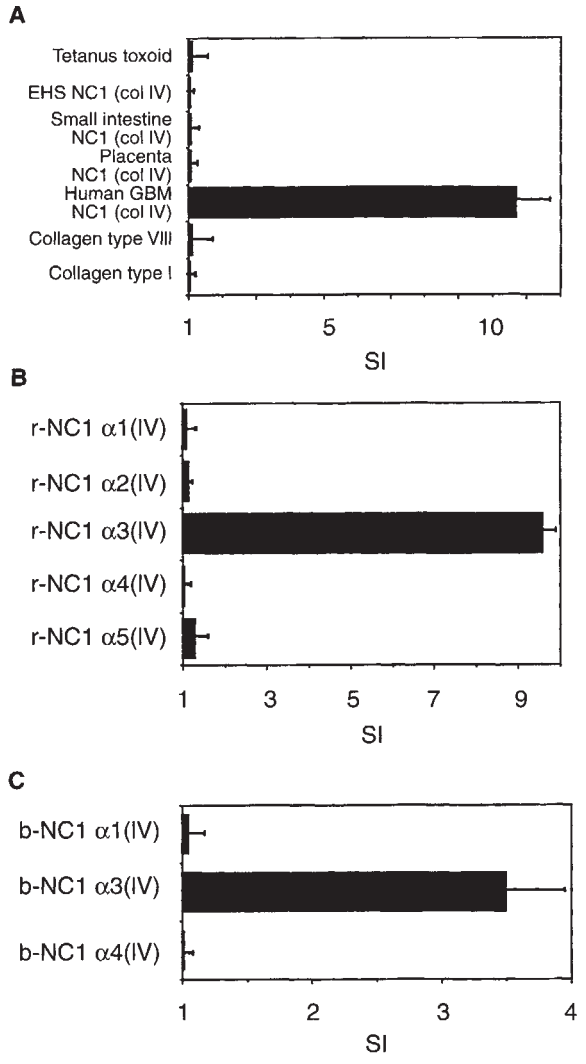

Fig. 3. (**A**) *The T-cell clone BF-C13 was examined for antigen induced T-cell proliferation in a ^{3}H-thymidine incorporation assay after 72 hours of culture.* Prior to testing, cells were cultured for 2 to 3 days without antigen, rIL-2 or autologous irradiated PBMC. The incorporated radioactivity was assessed by liquid scintillation counting. For better comparison, results are expressed as stimulation index (SI = cpm cells plus antigen/cpm cells without antigen). Type IV collagen NC1(IV) from EHS mouse sarcoma, tetanus toxoid, rat tail collagen type I and human placenta collagen typ VIII were used as negative control. All antigens were tested in a final concentration of 10^{-5} g/ml. (**B**) Reactivity of recombinant collagen type IV NC1 proteins on antigen induced proliferation. Among the five α-chains tested, only r-NC1-α3(IV) induced T-cell proliferation. (**C**) The mitogenic effects of bovine α1(IV), α3(IV) and α4(IV) NC1 proteins were tested. Again only bovine NC1-α3(IV) induced T-cell proliferation.

type IV collagen NC1(IV) as candidate autoantigen. Proliferating cells were subsequently cloned by limiting dilution. Three T-cell clones could be established that all responded to the C-terminal NC1 domain of GBM type IV collagen. Proliferation could also be induced in these T-cell clones by recombinant α3(IV) NC1 protein. In contrast, no response to NC1(IV) from human placenta, human small intestine and mouse EHS sarcoma cell lines could be induced. Also recombinant α1(IV), α2(IV), α4(IV) and α5(IV) proved to be ineffective. These results strongly suggest, that α3(IV) NC1 harbours the T-cell epitope.

For more detailed studies, the phenotype of T-cell clone BF-C13 was analyzed. Cells were $CD8^+$ and $CD45R0^+$. The CD45 molecule displays phosphotyrosine phosphatase activity and plays a critical role in regulating T-cell receptor (TcR) mediated signals. CD45 $R0^+$ populations are thought to be memory cells, but some of the CD45 $R0^+$ cells express markers characteristic of recently activated T-cells [23] The α/β^+ TcR of the T-cell clone BF-C13 expressed the Vβ 5.1 TcR chain on its surface. Generally, the T-cell receptor determines the repertoire of antigen-reactive T-cells and might be critically involved in the maintenance of peripheral tolerance to autoantigens [24]. The TcR Vβ chain in the established T-cell clone BF-C13 was primary sequenced to prove that the cells were of clonal origin. It is not known, whether the Vβ 5.1 chain is of importance for the development of the Goodpasture's syndrome. Since there are only few data on the T-cell receptor Vβ chain expression in Goodpasture's syndrome, it is impossible to say whether any chain is preferentially expressed in the state of disease.

MHC restriction analysis showed that response to antigen by the $CD8^+$ TCC BF-C13 was induced in conjunction with the class I allele HLA-A11. HLA DR2 association with Goodpasture's syndrome has previously been described [25] and was confirmed recently by using sequence-specific oligonucleotide probes [11, 26]. Especially the DR2 allele DRB1-1501 seems to be associated with disease (21 out of 23 patients). This HLA class II phenotype could also be demonstrated in the donor of the established T-cell clone. A close association with the class I allele HLA-A11 and the Goodpasture's syndrome has not been described. Furthermore, it remains unclear why all the clones raised were rather CD8 than CD4 positive. It might be due to the fact that cells were established after the initiation of therapy. Moreover, it cannot be excluded that it is the result of the cloning technique.

In order to characterize the target antigen of the T-cell clone BF-C13, we used different approaches. By comparing the proliferative response to two recombinant α3(IV) NC1 proteins, which differed in the number of residues, the region of interest could be mapped to the N-terminal area of the α3(IV) NC1 domain. We were able to show that strong proliferation could be induced by the full length rα3(IV) NC1 domain, while a truncated protein that lacks the N-terminal 26 amino acids was ineffective. We therefore speculated that likely in this area the T-cell epitope might be located. This hypothesis was further supported by testing several oligopeptides, designed with respect to major differences of the α3(IV) chain compared to the α1-α5(IV) chains. Since T-lymphocytes respond to self- or foreign peptides only in conjunction with MHC, additionally peptides with HLA-A11 specific motifs identified within the α3(IV) NC1 aminoacid sequence were included in our study. The HLA-A11 specific nonamer *GSPATWTTR* that is located within the N-terminal area of the α3(IV) NC1 domain induced proliferation in this particular T-cell clone. Therefore by combining the two different approaches, the N-terminal area of the α3(IV) NC1 domain is considered to be the T-cell epitope at least in this clone. The epitope is slightly different from the putative antibody-binding side [8].

In conclusion, our findings demonstrate that the three established T-cell clones are autoreactive to the α3(IV) NC1 domain of type IV collagen. For the first time a proliferating T-cell clone (BF-C13) could be established to localize the T-cell epitope at

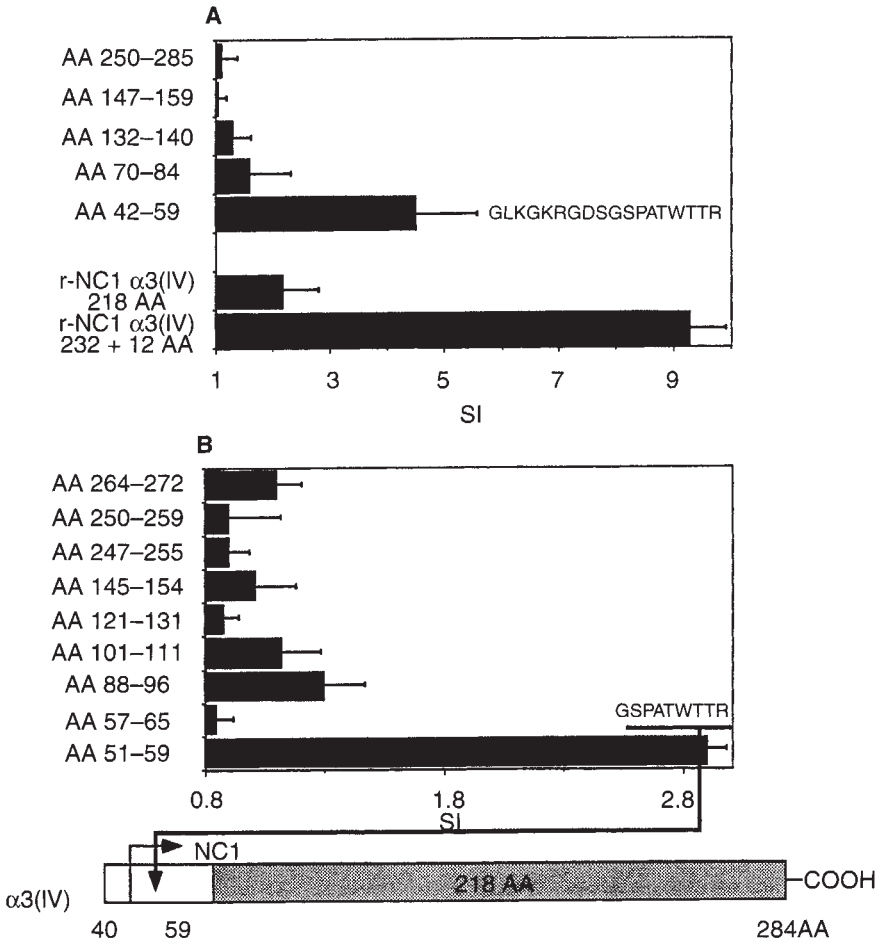

Fig. 4. *Epitope mapping.* (**A**) The T-cell clone BF-C13 was tested for oligopeptide induced proliferation by ^{3}H-thymidine incorporation assay after 72 hours of culture. Various peptides and two different r-NC1 α3(IV) proteins were tested. Only the full length NC1 α3(IV) protein (232 + 12 amino acids = AA) and the oligopeptide AA 42 to 59 stimulated the TCC. The incomplete r-NC1 α3(IV) protein (218 AA) lacks part of the N-terminal NC1 domain including the AA 42 to 59 and is thus ineffective in stimulating the T-cell clone. (**B**) Oligopeptides with HLA-A11 specific motifs were used to induce proliferation. Only the peptide 51 to 59 derived from the N-terminal NC1 domain stimulated T-cell proliferation.

least for this clone. Though these data have to be supported in further studies, they add substantially to our understanding of the pathogenesis of this disease. Goodpasture's syndrome therefore might be considered an autoimmune disorder in which activated T-cells participate apart from the well-known B-cell antibody response. This further suggests that drugs like cyclosporine A, which inhibit T-cell proliferation, more specifically might be of use in Goodpasture's syndrome. Moreover, future immunotherapy might include antigen-specific suppression of the immune response based on the knowledge of the antigenic epitope recognized by the T-cell receptor in patients with Goodpasture's syndrome. A comparable approach has already been used in multiple sclerosis [27] and in rheumatoid arthritis [28] where oral administration of the candidate autoantigen was used for induction of antigen specific tolerance.

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